Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications xxx (2006) xxx–xxx
BBRC
www.elsevier.com/locate/ybbrc

Increased efficacy of HIV-1 neutralization by antibodies at low CCR5 surface concentration

Vidita Choudhry [a,*], Mei-Yun Zhang [a,b], Ilia Harris [c], Igor A. Sidorov [a], Bang Vu [a], Antony S. Dimitrov [c], Timothy Fouts [c], Dimiter S. Dimitrov [a,*]

[a] *Protein Interactions Group, CCRNP, CCR, NCI-Frederick, NIH, Frederick, MD 21702, USA*
[b] *BRP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA*
[c] *Profectus BioSciences, Inc., 1450 South Rolling Road, Baltimore, MD 21227, USA*

Received 25 July 2006

Abstract

It has been observed that some antibodies, including the CD4-induced (CD4i) antibody IgG X5 and the gp41-specific antibody IgG 2F5, exhibit higher neutralizing activity in PBMC-based assays than in cell line based assays [J.M. Binley, T. Wrin, B. Korber, M.B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C.J. Petropoulos, D.R. Burton, Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies, J. Virol. 78 (2004) 13232–13252]. It has been hypothesized that the lower CCR5 concentration on the surface of the CD4 T lymphocytes compared to that on cell lines used for the neutralization assays could be a contributing factor to the observed differences in neutralizing activity. To test this hypothesis and to further elucidate the contribution of CCR5 concentration differences on antibody neutralizing activity, we used a panel of HeLa cell lines with well-defined and differential surface concentrations of CCR5 and CD4 in a pseudovirus-based assay. We observed that the CCR5 cell surface concentration but not the CD4 concentration had a significant effect on the inhibitory activity of X5 and several other CD4i antibodies including 17b and m9, as well as that of the gp41-specifc antibodies 2F5 and 4E10 but not on that of the CD4 binding site antibody (CD4bs), b12. The 50% inhibitory concentration (IC_{50}) decreased up to two orders of magnitude in cell lines with low CCR5 concentration corresponding to that in CD4 T cells used in PBMC-based assays (about 10^3 per cell) compared to cell lines with high CCR5 concentration (about 10^4 or more). Our results suggest that the CCR5 cell surface concentration could be a contributing factor to the high neutralizing activities of some antibodies in PBMC-based-assays but other factors could also play an important role. These findings could have implications for development of vaccine immunogens based on the epitopes of X5 and other CD4i antibodies, for elucidation of the mechanisms of HIV-1 neutralization by antibodies, and for design of novel therapeutic approaches.
Published by Elsevier Inc.

Keywords: HIV; Antibody; X5; 2F5; 4E10; CCR5; Envelope glycoprotein; Neutralization

Human immunodeficiency virus type I (HIV-1) enters cells by binding its envelope glycoprotein (Env, gp120-gp41) gp120 to CD4 and coreceptor (typically CCR5 or CXCR4) leading to activation of the gp41 fusion machinery and membrane fusion [1,2]. Antibodies can bind to the Env and interfere with the fusion process mostly by competing with CD4, coreceptors or fusion intermediates. Thus one can expect that Env-specific antibody inhibitory activity may depend on the cell surface receptor

* Corresponding authors. Addresses: Protein Interactions Group, CCRNP, CCR, NCI-Frederick, NIH, Building 320, Room 8, P.O. Box B, Miller Drive, Frederick, MD 21702-1201, USA (V.C.); Protein Interactions Group, CCRNP, CCR, NCI-Frederick, NIH, Building 469, Room 105, P.O. Box B, Miller Drive, Frederick, MD 21702-1201, USA (D.S.D.). Fax: +1 301 846 5598.
E-mail addresses: vchoudhry@ncifcrf.gov (V. Choudhry), dimitrov@ncifcrf.gov (D.S. Dimitrov).

0006-291X/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.bbrc.2006.07.163

concentration as has been also previously demonstrated for other HIV-1 entry inhibitors [3].

Fab X5 was selected from a human antibody phage library by using gp120-CD4-CCR5 complexes as an antigen, and showed a potent and broad neutralizing activity comparable on average to that of IgG b12 as tested with a panel of primary isolates from different clades [4]. It was initially proposed that similar to b12 the full antibody (in an IgG format) would exhibit even greater potency [4]. Because X5 binds better to gp120 complexed with CD4 than to gp120 alone, i.e., it is a CD4i (CD4 induced) antibody, it was reasonable to hypothesize that vaccine immunogens able to elicit X5-like antibodies, e.g., complexes of gp120 with CD4 or CD4 mimics, could have potential as AIDS vaccines. However, later it was found that for most of the tested isolates IgG X5, which is bivalent but of relatively large size, is less potent than Fab X5, which has smaller size. The most potent antibody format for most but not all isolates was the scFv X5 which is the smallest ([5], and unpublished data); this finding dashed hopes to use the X5 epitope as a template for design of vaccine immunogens but still offered the possibility for use of its epitope as a target for potent inhibitors of small size. Indeed, based on scFv X5, a very potent antibody in a scFv format, m9, was developed [6]; however, an IgG-like format of this antibody also showed on average lower inhibitory activity compared to the scFv format (Zhang et al., unpublished data). Recently, it was reported that IgG X5 neutralizes significantly higher number of primary isolates (7 vs 1) in a PBMC-based neutralization assay compared to a cell line-based assay, and proposed that one of the factors that could contribute to these differences is related to differences in the surface concentrations of receptors molecules [7]. In the same report it was also described that 2F5 also exhibits higher neutralizing activity in a PBMC-based assay compared to a cell line-based assay although the difference between the two assays was significantly smaller than for X5; interestingly the other gp41-specific antibody tested in the same study exhibited much broader and higher neutralizing activity in the cell line/pseudovirus-based assay than in the PBMC-based assay.

It was previously found that the coreceptor surface concentrations can reach up to 10^5 antibody binding sites (ABS) for some cell lines, while they do not exceed 10^4 ABS for CD4 T lymphocytes [8–12]. Although activation of T cells can lead to an increase in the receptor and coreceptor concentrations, the CCR5 surface concentration is relatively low in PBMCs unless specific activation aimed to induce high CCR5 concentration is used [13]. To elucidate the mechanism of CCR5 concentration on the inhibitory activity of CD4i antibodies and other antibodies, we used a panel of HeLa cell lines with well-defined differential surface concentrations of CCR5 and CD4 in a pseudovirus neutralization assay. Here we report significant effect of the CCR5 cell surface concentration on the inhibitory activity of X5 [4,14–16] and several other antibodies.

Materials and methods

Cells and antibodies. HeLa cell lines, expressing different levels of CCR5 and CD4 [17], were gift from D. Kabat (Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, Oregon). TZM-bl cell line was obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health (catalog No. 8129). HEK 293T cells were purchased from American Type Culture Collection (Manassas, VA). All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum. PBMCs were isolated from a healthy volunteer, stimulated with PHA-P (Sigma, St. Louis, MO) for 3 days, and grown in 20 U/ml of IL-2 (BD Pharmingen, San Jose, CA) for seven additional days. The human mAbs IgG b12 was a gift from D. Burton (The Scripps Research Institute, La Jolla, CA), and IgG 17b from J. Robinson (Tulane University Medical Center, New Orleans, LA); mAbs IgG X5, scFv m9, m16 (scFv and IgG)

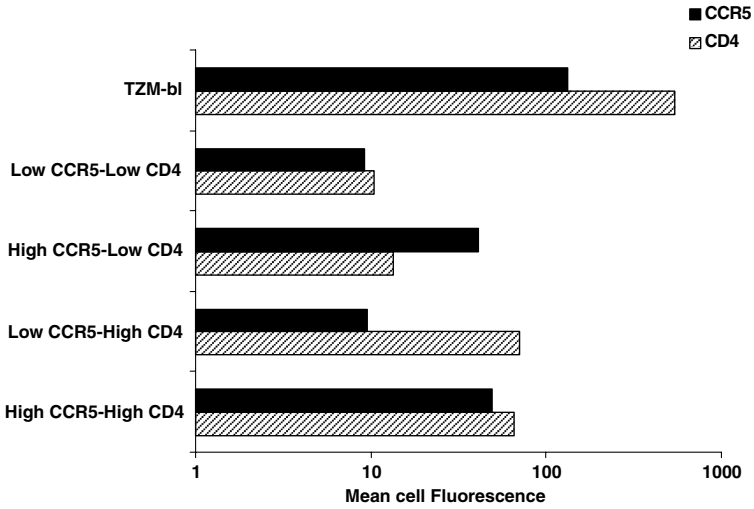

Fig. 1. Cell surface expression of CD4 and CCR5 in the four cell lines used for measurement of antibody neutralization. The immunostaining of different HeLa and TZM-bl cell lines was performed with FITC-conjugated anti-CD4 and PE-conjugated anti-CCR5 antibodies by flow cytometry. The mean fluorescence values for each cell line are shown.

Please cite this article as: Vidita Choudhry et al., Increased efficacy of HIV-1 neutralization by antibodies at low ..., Biochemical and Biophysical Research Communications (2006), doi:10.1016/j.bbrc.2006.07.163.

and scFv 17b were produced in our laboratory; the human monoclonal antibodies 2F5 and 4E10 were gift from H. Katinger (Institute of Applied Microbiology, University for Agricultural Sciences, Vienna, Austria). All the IgGs (human mAbs) used in the present study were of IgG1 subtype. The following antibodies were purchased: PE-conjugated mouse anti-CCR5 (2D7 and 3A9) from BD Pharmingen (San Jose, CA) and FITC-conjugated mouse anti-CD4 (RPA-T4) from BioLegend (San Diago, CA). Isotype control FITC and PE-conjugated mouse IgG_{2a},κ antibodies were purchased from BD Phamingen (San Jose, CA).

Flow cytometry. Twelve different HeLa cell lines [17] and PBMCs were analyzed by flow cytometry. HeLa cells were harvested with dissociation buffer from GIBCO/BRL (Grand Island, NY), centrifuged at 450*g*, and resuspended at 10^7 cells/ml. PE-conjugated mouse anti-CCR5 (2D7) and FITC-conjugated mouse anti-CD4 (RPA-T4) were added to 100 μl (10^6 cells) sample at 1:5 dilution. Cells were incubated at 4 °C for 1 h, washed twice with PBS containing 0.2% BSA, and resuspended in PBS to be read by FACScan Flow Cytometer (BD Bioscience, San Jose, CA) at 10,000 events/sample with respect to unlabeled cells. Mean cell fluorescence was calculated using the CellQuest software (BD Bioscience, SanJose, CA). PBMCs and TZM-bl were measured similarly by using PE-conjugated mouse anti-CCR5 (3A9) and FITC-conjugated mouse anti-CD4 (RPA-T4) antibodies. PE

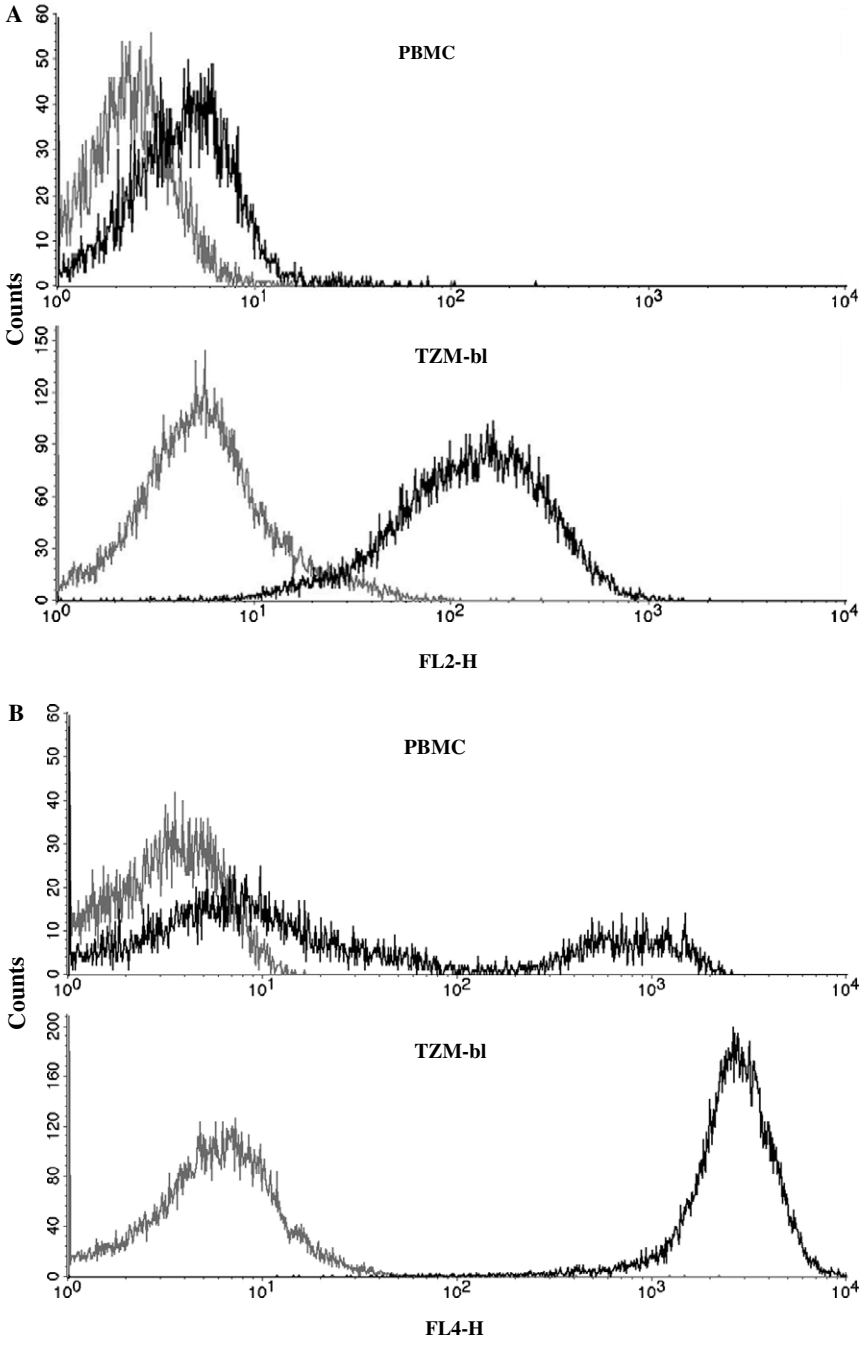

Fig. 2. Comparison of the CCR5 expression levels on the surface of PBMCs and TZM bl cells. The cells were double stained with FITC-conjugated anti-CD4 (RPA-T4) and PE-conjugated anti-CCR5 (3A9) antibodies. The histograms show ungated data for the uniform TZM-bl cells and T-cells gated population data for the PBMCs. (A) CCR5 cell surface expression (B) CD4 cell surface expression, staining of PBMCs and TZM-bl cells with isotype control antibodies is shown in gray color.

Please cite this article as: Vidita Choudhry et al., Increased efficacy of HIV-1 neutralization by antibodies at low ..., Biochemical and Biophysical Research Communications (2006), doi:10.1016/j.bbrc.2006.07.163.

and FITC-conjugated mouse IgG_{2a},κ were used as isotype controls for CCR5 and CD4, respectively.

Assay for virus infectivity. Viruses pseudotyped with Envs from HIV-1 primary isolates representing HIV-1 group M, clades A–E [18] were used in this study. Briefly, pseudotyped viruses were prepared by cotransfection of 70–80% confluent 293T cells with pNL4-3.luc.E-R- and pSV7d-env plasmid using the PolyFect transfection reagent, according to manufacturer's instruction (Qiagen GmbH, Hilden). Pseudotyped viruses were obtained after 24 h by centrifugation and filtration through 0.45 μm filters and mixed with different concentrations of antibodies for 30 min at 37 °C, then added to 1.5×10^4 HeLa-CD4/CCR5 cells. Luminesence was measured after 3 days, using the Bright-Glo Luciferase Assay System (Promega, Madison, WI) and a LumiCount microplate luminometer (Turner Designs). Mean relative light units (RLU) for triplicate wells were determined. Percentage inhibition was calculated by the following formula: (1 – average RLU of antibody-containing wells/average RLU of virus-only wells) × 100. IC_{50} of neutralization was assigned for the antibody concentration at which 50% neutralization was observed.

Cell–cell fusion assay. The β-gal reporter gene cell–cell fusion assay has been previously described [19]. Briefly, HIV-1 Env-CD4-mediated cell fusion was performed by incubation of 10^5 293T cells expressing Env (after infection with recombinant vaccinia virus vCB21R, encoding the *lacZ* gene under the control of the T7 promoter) mixing with 10^5 HeLa cells expressing different concentrations of CCR5 and CD4 (after infection with recombinant vaccinia viruses vTF7-3, encoding T7 RNA polymerase) for 2 h at 37 °C. The inhibitory effect of scFv m9 was evaluated by mixing the Env expressing cells with different concentrations of antibody for 30 min at 37 °C and then performing the fusion assay. β-Gal activity was quantified by a colorimetric assay that measures the optical density at 595 nm.

Results

To elucidate the role of receptor surface concentrations for antibody neutralizing activity we used HeLa cell lines, previously developed by Kabat and co-workers [17], with well-defined surface concentrations of CD4 and CCR5. To confirm and select clones on the basis of significant differences in the levels of cell surface concentrations of CD4 and CCR5, 12 different cell lines were analyzed by flow cytometry. We selected four cell lines, which resemble T lymphocytes and cell lines used in neutralization assays with respect to the surface concentrations of CD4 and CCR5 (Fig. 1). Two of these cell lines, High CCR5–High CD4 (clone JC.53) and Low CCR5–High CD4 (clone JC.10), have high CD4 surface concentration of about 4×10^5 molecules per cell, while the other two cell lines, High CCR5–Low CD4 (clone RC.49) and Low CCR5–Low CD4 (clone RC.55), have low CD4 surface concentrations (approximately 10^4 molecules/cell) [17]. The Low CCR5–High CD4 and Low CCR5–Low CD4 cell lines have relatively low CCR5 concentrations of 2.0×10^3 and 2.1×10^4 molecules per cell, respectively, and High CCR5–High CD4 and High CCR5–Low CD4 cell lines have high CCR5 surface concentrations: 1.3×10^5 and 8.5×10^4, respectively [17]. These major differences in

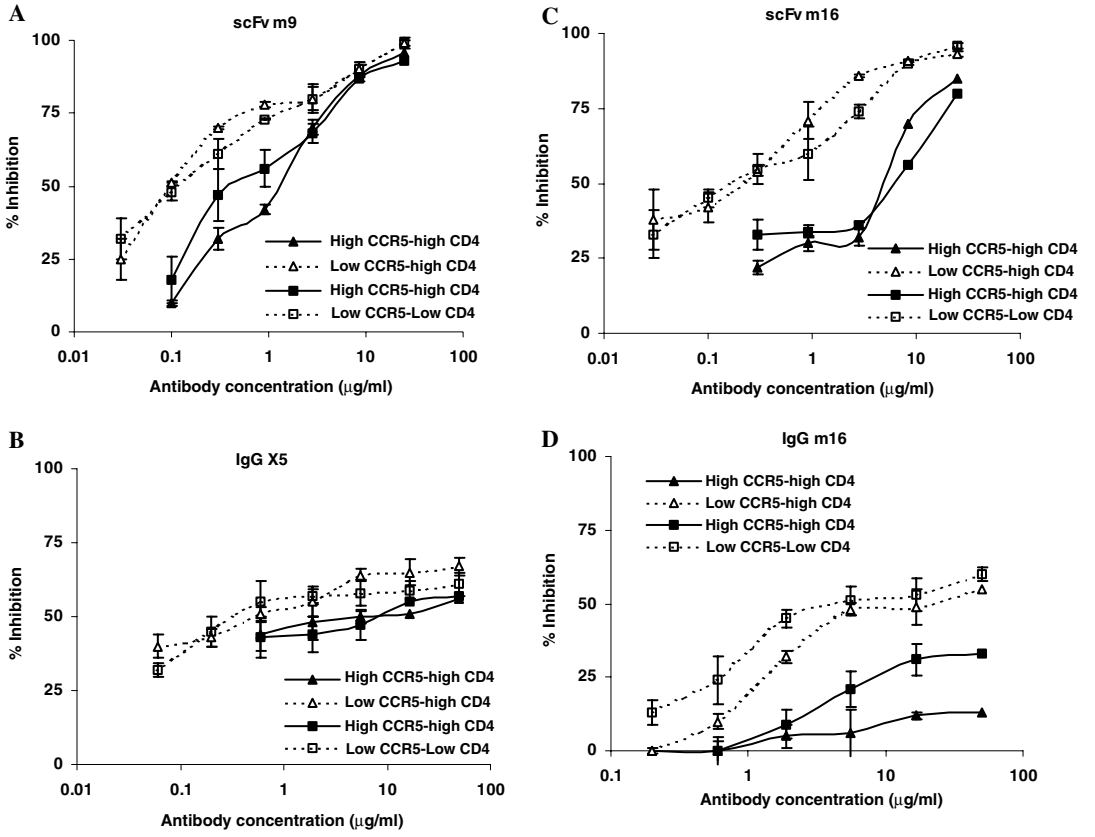

Fig. 3. Neutralizing activity of CD4i antibodies against primary HIV-1 isolate from clade B (Bal) in a pseudovirus-based assay. Neutralization assays were carried out in triplicate wells by preincubation of serial dilutions of (A) scFv m9, (B) IgG X5, (C) scFv m16, and (D) IgG m16 with pseudotyped viruses for 30 min at 37 °C followed by infection of 1.5×10^4 HeLa cells. Luminescence was measured after 3 days, the mean luminescence readings for triplicate wells and standard deviations were determined; the percentage inhibition of luciferase activity is presented as a measure of the antibody inhibitory activity.

Please cite this article as: Vidita Choudhry et al., Increased efficacy of HIV-1 neutralization by antibodies at low ..., Biochemical and Biophysical Research Communications (2006), doi:10.1016/j.bbrc.2006.07.163.

receptor and coreceptor surface concentrations still existed after culturing and selection of clones as measured by flow cytometry, although the CCR5 surface concentration in Low CCR5–High CD4 and Low CCR5–Low CD4 cell lines appears about the same (Fig. 1). TZM-bl cells, which are frequently used for cell line/pseudovirus-based assays, had higher CCR5 surface concentration than selected HeLa cells (Fig. 1). Similarly, the CCR5 concentration on the surface of TZM-bl cells was significantly higher than on PBMCs (Fig. 2A), however, CD4 surface concentration was about the same (Fig. 2B). These results indicate that CCR5 concentration on the surface of PBMCs was comparable to those of selected Low CCR5–High CD4 and Low CCR5–Low CD4 cell lines.

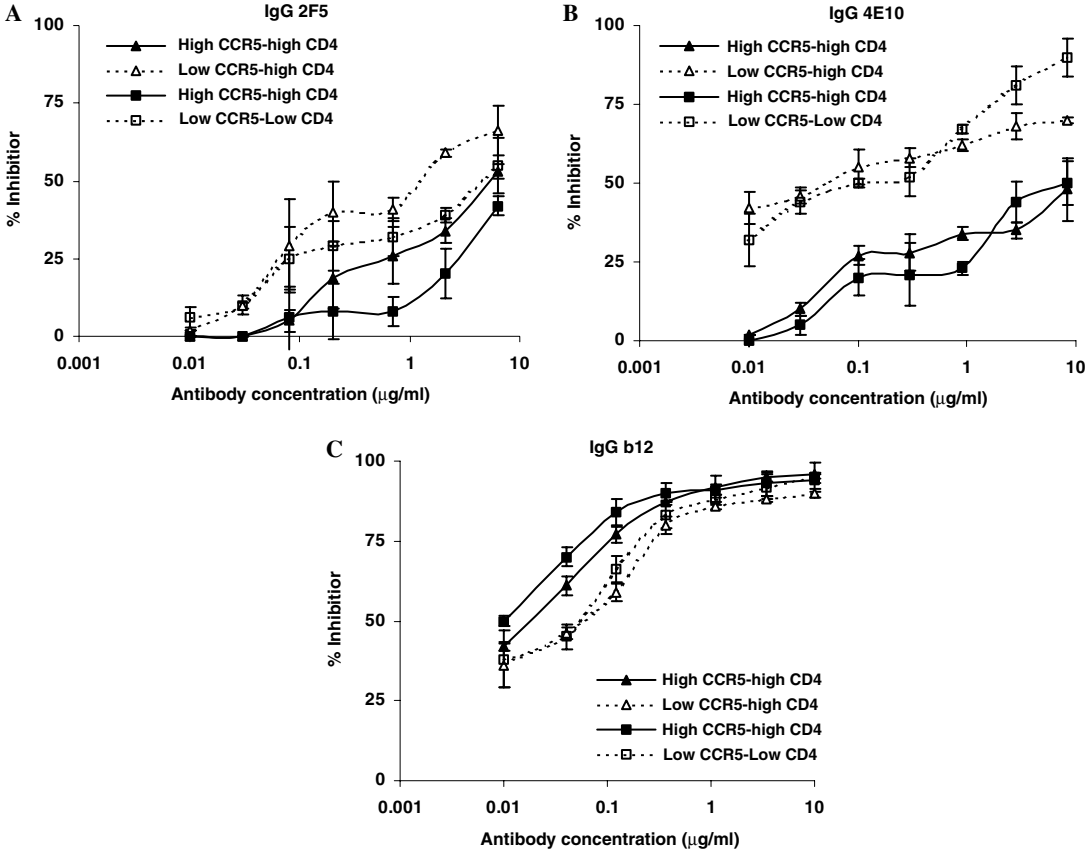

Fig. 4. Neutralizing activity of CD4bs and anti-gp41 antibodies against primary HIV-1 isolate from clade B (Bal) in a pseudovirus-based assay. The percentage inhibition of luciferase activity is presented as a measure of the antibody inhibitory activity (see legend of Fig. 2) of gp41-specific antibodies, (A) IgG 2F5 and (B) IgG 4E10 and (C) for CD4bs antibody IgG b12.

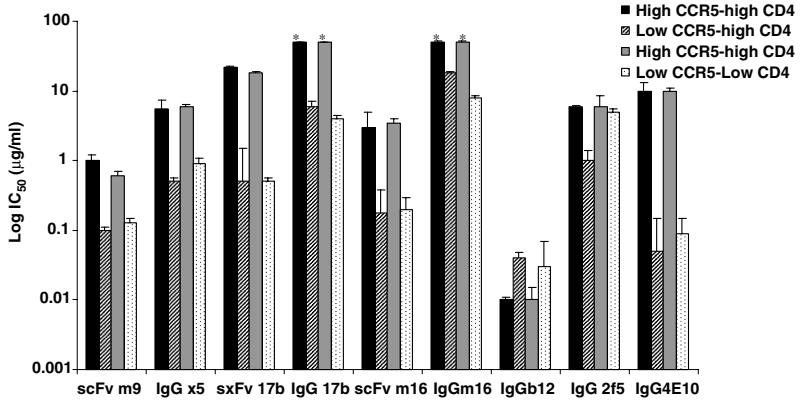

Fig. 5. Neutralization of HIV-1 Env (Bal) by antibodies. Inhibitory activity of CD4i (X5, m9, 17b, m16), CD4bs (b12), and gp41-specific antibodies (2F5 and 4E10) was determined by a pseudovirus-based assay for primary isolate of HIV-1 from clade B (Bal). IC_{50} value (μg/ml) was assigned to the antibody concentration at which 50% neutralization was observed. *Indicates the highest tested concentration of IgG 17b and IgG m16 at which 50% inhibition could not be achieved. The mean of three measured IC_{50}s (in μg/ml) for each antibody and the standard deviations are presented.

Please cite this article as: Vidita Choudhry et al., Increased efficacy of HIV-1 neutralization by antibodies at low ..., Biochemical and Biophysical Research Communications (2006), doi:10.1016/j.bbrc.2006.07.163.

We found that IgG X5 and m9, which is a derivative of X5 in a scFv format with an improved potency and breadth of neutralization [6], exhibited significantly higher (10-fold decrease in IC_{50}) neutralization activity when tested in cells with low surface concentration of CCR5 compared to those with high CCR5 concentration; on average the CD4 concentration did not significantly affect the antibody neutralizing activity for these cells (Fig. 3A and B). There was no significant difference in IC_{50} for cells with about the same CCR5 concentration. Similarly, even larger effects of up to two orders of magnitude were observed for another CD4i antibody, m16 [20], as scFv and IgG (Fig. 3C and D). A significant change in IC_{50} was also observed for the gp41-specific antibody IgG 2F5 for Low CCR5 cells compared to High CCR5 cells (Fig. 4A). The inhibitory activity of the other broadly HIV-1 neutralizing gp41-specific antibody, IgG 4E10, was even more significantly affected by the CCR5 concentration than for 2F5 similar to the effect observed for IgG X5 (Fig. 4B). Interestingly, the neutralizing activity of the CD4bs antibody IgG b12 was higher for High CCR5 cells compared to Low CCR5 cells at the same surface concentration of CD4 (Fig. 4C). Fig. 5 shows a summary of the results for these and other antibodies neutralizing the Bal isolate from clade B.

We also observed about the same or even larger differences in the inhibitory activity of IgG X5 and scFv m9 for cells with different CCR5 surface concentrations infected by other primary isolates from different clades (Fig. 6 and Table 1). In contrast, the IC_{50} of IgG b12 was not affected by the CCR5 concentration to any significant extent for all isolates tested. Similarly, the IC_{50} of the gp41-specific antibody IgG 2F5 did not depend significantly on the CCR5 concentration for most isolates although for some there was a trend for an increased inhibitory activity at low CCR5 concentration. This trend was significant for the other gp41-specific antibody, IgG 4E10, which did exhibit higher neutralizing activity for cells with lower CCR5 cell surface concentration. In general, the CD4i antibodies exhibited up to two orders of magnitude higher neutralizing activity (lower IC_{50}) for all tested isolates when infecting cells with low CCR5 surface concentrations (Low CCR5–High CD4, Low CCR5–Low CD4) compared to cells with high CCR5 surface concentrations. We could not determine IC_{50} of neutralization for some of the isolates (Table 1), however, the percentage inhibition observed at the highest concentration of the antibody tested did reflect similar trend, as the percentage inhibition was increased for cells with low CCR5 concentration in comparison to cells with High CCR5 concentration.

We have also used a cell–cell fusion assay to further determine the effect of CCR5 cell surface concentration on the inhibitory activity of the CD4i antibody scFv m9.

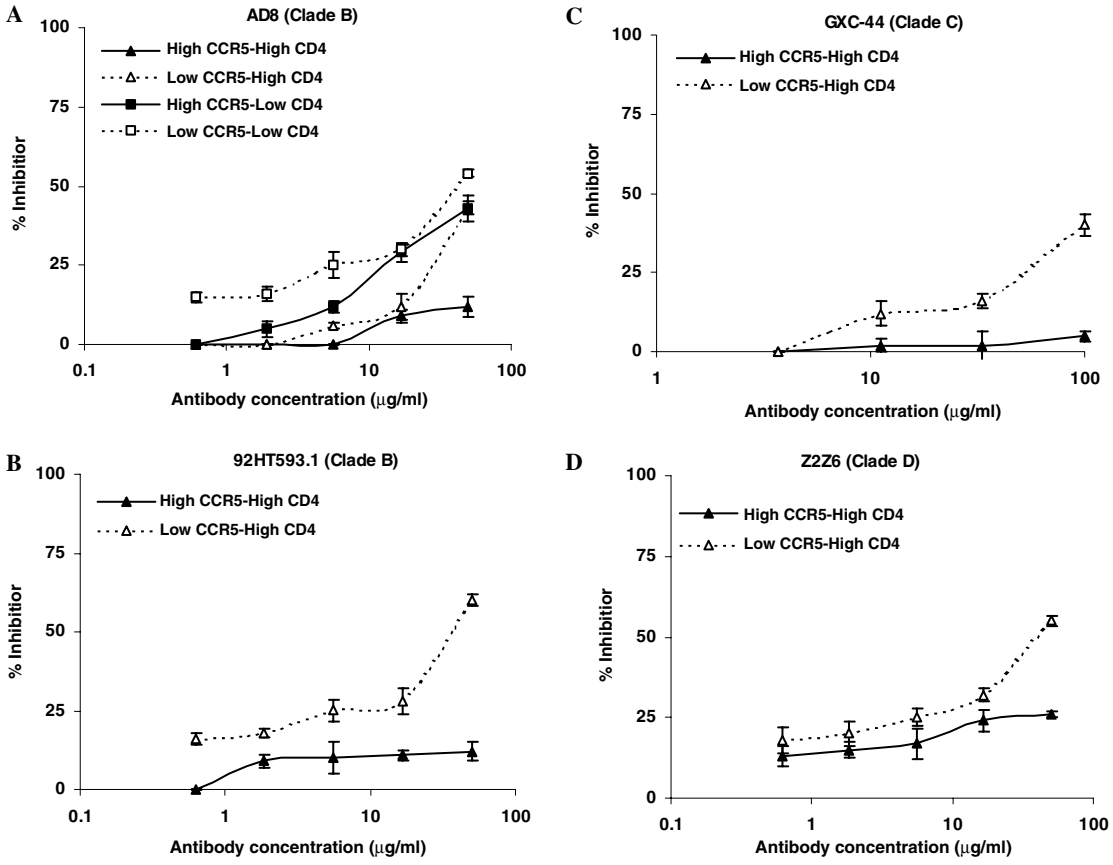

Fig. 6. Neutralization of HIV-1 isolates from different clades by IgG X5. The percentage inhibition of luciferase activity is presented as a measure of the IgG X5 neutralizing activity (see legend of Fig. 2) against the indicated four isolates from four different clades.

Please cite this article as: Vidita Choudhry et al., Increased efficacy of HIV-1 neutralization by antibodies at low ..., Biochemical and Biophysical Research Communications (2006), doi:10.1016/j.bbrc.2006.07.163.

Table 1
Neutralization of HIV-1 primary isolates from different clades by IgG X5 and other antibodies in an assay based on cell lines with different surface concentrations of CD4 and CCR5

HIV-1 clade	Antibody	High CCR5–High CD4	Low CCR5–High CD4
92UG037.8 (Clade A)	scFv m9	>25 (25%)	4
	IgG b12	>25 (44%)	>25 (41%)
	IgG 2F5	0.3	0.1
	IgG 4E10	0.9	0.2
Bal (Clade B)	scFv m9	1	0.1
	IgG X5	5.5	0.5
	scFv 17b	22	0.5
	IgG 17b	>50 (32%)	6
	scFv m16	3	0.18
	IgG m16	>50 (13%)	18
	IgG b12	0.01	0.04
	IgG 2F5	6	1
	IgG 4E10	10	0.05
AD8 (Clade B)	ScFv m9	15	2.5
	IgG X5	>50 (12%)	>50 (43%)
	IgG b12	0.5	0.5
	IgG 2F5	>25 (45%)	22
	IgG 4E10	>25 (28%)	25
92HT593.1 (Clade B)	scFv m9	>25 (37%)	1.5
	IgG X5	>50 (12%)	40
	scFv 17b	20	8
	IgG 17b	>50 (35%)	>50 (43%)
	scFv m16	>25 (14%)	2
	IgG m16	>50 (10%)	>50 (40%)
	IgG b12	2	1.2
	IgG 2F5	4	0.8
	IgG 4E10	10	2
GXC-44 (Clade C)	scFv m9	3	0.3
	IgG X5	>100 (5%)	>100 (40%)
	IgG b12	>25 (25%)	>25 (22%)
	IgG 2F5	>25 (9%)	>25 (8%)
	IgG 4E10	25	18
Z2Z6 (Clade D)	scFv m9	3	0.9
	IgG X5	>50 (26%)	35
	IgG b12	5	4
	IgG 2F5	25	20
	IgG 4E10	>25 (42%)	15
CM243 (Clade E)	scFv m9	>25 (19%)	>25 (34%)
	IgG X5	>25 (17%)	>50 (30%)
	IgG b12	>25 (27%)	>25 (30%)
	IgG 2F5	2.8	1.5
	IgG 4E10	2	1

Antibodies at various concentrations were mixed with viruses pseudotyped with the Env from different clades of HIV-1 for 30 min at 37 °C and mixed with cells expressing high (High CCR5–High CD4) or low (Low CCR5–High CD4) CCR5 concentrations in triplicate wells. The mean luminescence readings were determined 3 days later and IC_{50} (μg/ml) was calculated at the antibody concentration at which 50% neutralization was observed. The following antibodies were used: the CD4i antibodies X5, m9, 17b, and m16 in different formats; the CD4 binding site antibody b12, and the gp41-specific antibodies 2F5 and 4E10 in an IgG format. The mean of three measured IC_{50}s for each antibody/virus/cell combination is presented (in μg/ml); the standard deviation was on average 5% and did not exceed 20%, the numbers in parentheses are the percentage neutralization measured at those concentrations used.

We observed inhibition of fusion mediated by Env of primary isolate from clade B (Bal) with a twofold higher potency for Low CCR5–High CD4 cell line in comparison to High CCR5–High CD4 ones. Similarly up to fourfold decrease in IC_{50} was observed for Low CCR5–Low CD4 cells in comparison to High CCR–Low CD4 cells (Fig. 7A). We also observed up to threefold higher inhibition of fusion for another clade B primary isolate of HIV-1 (AD8) by scFv m9 for Low CCR5–High CD4 cell line in comparison to High CCR5–High CD4 cells (Fig. 7B). However, we did not observe significant difference in the IC_{50} of fusion inhibition for cell lines with High CCR5–Low CD4 and Low CCR5–Low CD4, which could be due to the very slow fusion rate.

Please cite this article as: Vidita Choudhry et al., Increased efficacy of HIV-1 neutralization by antibodies at low ..., Biochemical and Biophysical Research Communications (2006), doi:10.1016/j.bbrc.2006.07.163.

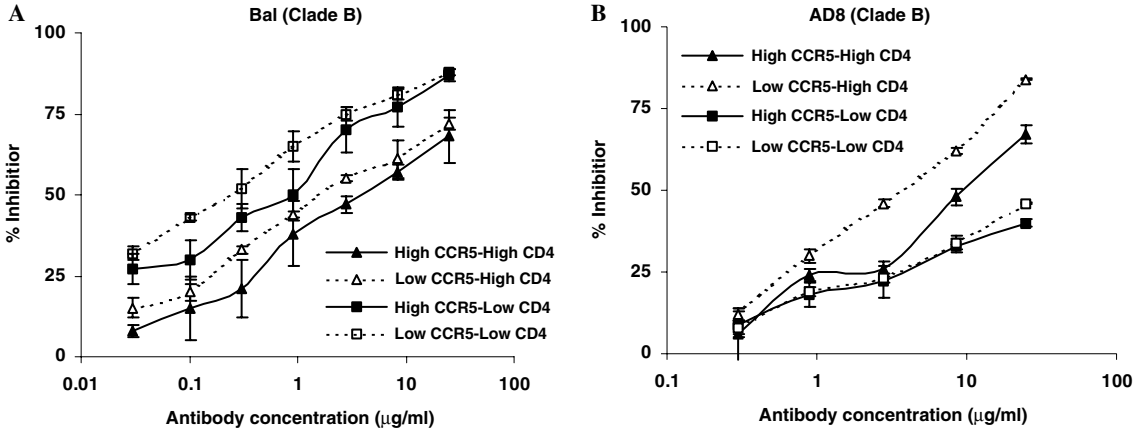

Fig. 7. Inhibition of cell–cell fusion by scFv m9. Inhibitory activity of scFv m9 was studied by incubating the serially diluted antibody with Env expressing cells for 30 min at 37 °C before mixing with HeLa cells. The inhibitory effect of m9 was evaluated by mixing the Env expressing cells with m9 for 30 min at 37 °C and then performing the fusion assay. Percentage inhibition of β-gal activity is presented as a measure of antibody inhibitory activity for (A) Env Bal and (B) Env AD8.

Discussion

The mechanism of the high neutralizing activity of CD4i antibodies and some gp41-specific antibodies including 4E10 in cells with low CCR5 surface concentration could include factors related to the slow fusion kinetics of cells expressing low CCR5 concentrations with the virus or with cells expressing the HIV-1 Env as demonstrated for another fusion inhibitor T20 [3]. Whatever the mechanism is, these findings have several important implications. First, CD4 T lymphocytes express relatively low levels of CCR5 [21] and therefore X5 including IgG X5 could exhibit potent neutralizing activity in vivo, which is in agreement with the finding of significantly higher neutralizing activity of IgG X5 in a PBMC-based assay compared to a cell line-based assay [7]. One can also speculate that in infected humans neutralizing antibodies could prevent more efficiently cells expressing low levels of CCR5 that could lead to coreceptor switch because the rate of entry in such cells could be slow although we do not know whether similar dependences on the other major coreceptor CXCR4 exist. Second, this finding could have implications for understanding of the mechanisms of HIV-1 neutralization by antibodies. The very fact that the extent of neutralization is dependent on the cell surface concentration of a coreceptor could imply that the number of antibody molecules bound to a virus particle that are required to neutralize could vary in dependence on the coreceptor concentration; this could be related to the post CD4-binding component of the mechanism of neutralization by the CD4i and gp41-specific antibodies. Third, X5 is a potent broadly CD4 induced (CD4i) HIV-1 neutralizing antibody in its Fab and scFv formats but less potent as a full antibody in an IgG format, and the potential use of its epitope as a template for design of vaccine immunogens has been debated. If the IgG X5 activity in vivo is at least as high as in cells with low CCR5 concentrations in vitro, its epitope could be used in the design of vaccine immunogens aimed at eliciting X5-like antibodies, although only further experiments in animal models and in humans can provide definite evidence for the utility of the X5 (or even better m9) epitope as a template of a successful vaccine immunogen. Fourth, one can reason that X5 and other antibodies could synergize with inhibitors aimed at decreasing the CCR5 surface concentration, including anti-CCR5 antibodies and small molecules as rapamycin [22]. Therefore these findings could have implications for elucidation of the mechanisms of HIV-1 neutralization by antibodies, development of vaccine immunogens based on the epitopes of X5 and X5-like antibodies, and design of novel therapeutic approaches.

Acknowledgments

We thank David Kabat for kindly providing the panel of the HeLa cell lines Hana Golding—for interesting discussions that stimulated us to perform this study. We also thank Gabriela Stiegler, and Herman Katinger—2F5 and 4E10, Dennis Burton—b12, James Robinson—17b, and Xiaodong Xiao and Luke Stockwin for help with the experiments. This research was supported by the IATAP of the NIH, by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, by federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-CO-12400, CRADA between National Cancer Institute and Profectus BioSciences, Inc., and the Gates Foundation to D.S.D.

References

[1] D.S. Dimitrov, Virus entry: molecular mechanisms and biomedical applications, Nat. Rev. Microbiol. 2 (2004) 109–122.
[2] A.E. Smith, A. Helenius, How viruses enter animal cells, Science 304 (2004) 237–242.

[3] J.D. Reeves, S.A. Gallo, N. Ahmad, J.L. Miamidian, P.E. Harvey, M. Sharron, S. Pohlmann, J.N. Sfakianos, C.A. Derdeyn, R. Blumenthal, E. Hunter, R.W. Doms, Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics, Proc. Natl. Acad. Sci. USA 99 (2002) 16249–16254.

[4] M. Moulard, S.K. Phogat, Y. Shu, A.F. Labrijn, X.D. Xiao, J.M. Binley, M.Y. Zhang, I.A. Sidorov, C.C. Broder, J. Robinson, P.W.H.I. Parren, D.R. Burton, D.S. Dimitrov, Broadly cross-reactive HIV-1-neutralizing human monoclonal Fab selected for binding to gp120-CD4-CCR5 complexes, Proc. Natl. Acad. Sci. USA 99 (2002) 6913–6918.

[5] A.F. Labrijn, P. Poignard, A. Raja, M.B. Zwick, K. Delgado, M. Franti, J. Binley, V. Vivona, C. Grundner, C.C. Huang, M. Venturi, C.J. Petropoulos, T. Wrin, D.S. Dimitrov, J. Robinson, P.D. Kwong, R.T. Wyatt, J. Sodroski, D.R. Burton, Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1, J. Virol. 77 (2003) 10557–10565.

[6] M.Y. Zhang, Y. Shu, D. Rudolph, P. Prabakaran, A.F. Labrijn, M.B. Zwick, R.B. Lal, D.S. Dimitrov, Improved breadth and potency of an HIV-1-neutralizing human single-chain antibody by random mutagenesis and sequential antigen panning, J. Mol. Biol. 335 (2004) 209–219.

[7] J.M. Binley, T. Wrin, B. Korber, M.B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C.J. Petropoulos, D.R. Burton, Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies, J. Virol. 78 (2004) 13232–13252.

[8] G. Meng, M.T. Sellers, M. Mosteller-Barnum, T.S. Rogers, G.M. Shaw, P.D. Smith, Lamina propria lymphocytes, not macrophages, express CCR5 and CXCR4 and are the likely target cell for human immunodeficiency virus type 1 in the intestinal mucosa, J. Infect. Dis. 182 (2000) 785–791.

[9] J. Olsson, M. Poles, A.L. Spetz, J. Elliott, L. Hultin, J. Giorgi, J. Andersson, P. Anton, Human immunodeficiency virus type 1 infection is associated with significant mucosal inflammation characterized by increased expression of CCR5, CXCR4, and beta-chemokines, J. Infect. Dis. 182 (2000) 1625–1635.

[10] P.A. Anton, J. Elliott, M.A. Poles, I.M. McGowan, J. Matud, L.E. Hultin, K. Grovit-Ferbas, C.R. Mackay, I.S.Y. Chen, J.V. Giorgi, Enhanced levels of functional HIV-1 co-receptors on human mucosal T cells demonstrated using intestinal biopsy tissue, AIDS 14 (2000) 1761–1765.

[11] M.A. Nokta, X.D. Li, J. Nichols, M. Mallen, A. Pou, D. Asmuth, R.B. Pollard, Chemokine/CD4 receptor density ratios correlate with HIV replication in lymph node and peripheral blood of HIV-infected individuals, AIDS 15 (2001) 161–169.

[12] F. Hladik, G. Lentz, E. Delpit, A. McElroy, M.J. McElrath, Coexpression of CCR5 and IL-2 in human genital but not blood T cells: implications for the ontogeny of the CCR5+ Th1 phenotype, J. Immunol. 163 (1999) 2306–2313.

[13] X. Xiao, L. Wu, T.S. Stantchev, Y.R. Feng, S. Ugolini, H. Chen, Z. Shen, J.L. Riley, C.C. Broder, Q.J. Sattentau, D.S. Dimitrov, Constitutive cell surface association between CD4 and CCR5, Proc. Natl. Acad. Sci. USA 96 (1999) 7496–7501.

[14] R. Darbha, S. Phogat, A.F. Labrijn, Y. Shu, Y. Gu, M. Andrykovitch, M.Y. Zhang, R. Pantophlet, L. Martin, C. Vita, D.R. Burton, D.S. Dimitrov, X. Ji, Crystal structure of the broadly cross-reactive HIV-1-neutralizing Fab X5 and fine mapping of its epitope, Biochemistry 43 (2004) 1410–1417.

[15] C.C. Huang, M. Venturi, S. Majeed, M.J. Moore, S. Phogat, M.Y. Zhang, D.S. Dimitrov, W.A. Hendrickson, J. Robinson, J. Sodroski, R. Wyatt, H. Choe, M. Farzan, P.D. Kwong, Structural basis of tyrosine sulfation and VH-gene usage in antibodies that recognize the HIV type 1 coreceptor-binding site on gp120, Proc. Natl. Acad. Sci. USA 101 (2004) 2706–2711.

[16] C.C. Huang, M. Tang, M.Y. Zhang, S. Majeed, E. Montabana, R.L. Stanfield, D.S. Dimitrov, B. Korber, J. Sodroski, I.A. Wilson, R. Wyatt, P.D. Kwong, Structure of a V3-containing HIV-1 gp120 core, Science 310 (2005) 1025–1028.

[17] E.J. Platt, K. Wehrly, S.E. Kuhmann, B. Chesebro, D. Kabat, Effects of CCR5 and CD4 cell surface concentrations on infections by macrophagetropic isolates of human immunodeficiency virus type 1, J. Virol. 72 (1998) 2855–2864.

[18] F. Cham, P.F. Zhang, L. Heyndrickx, P. Bouma, P. Zhong, H. Katinger, J. Robinson, G.G. van der, G.V. Quinnan Jr., Neutralization and infectivity characteristics of envelope glycoproteins from human immunodeficiency virus type 1 infected donors whose sera exhibit broadly cross-reactive neutralizing activity, Virology 347 (2006) 36–51.

[19] C.C. Broder, E.A. Berger, Fusogenic selectivity of the envelope glycoprotein is a major determinant of human immunodeficiency virus type 1 tropism for CD4+ T-cell lines vs. primary macrophages, Proc. Natl. Acad. Sci. USA 92 (1995) 9004–9008.

[20] M.Y. Zhang, Y. Shu, I. Sidorov, D.S. Dimitrov, Identification of a novel CD4i human monoclonal antibody Fab that neutralizes HIV-1 primary isolates from different clades, Antiviral Res. 61 (2004) 161–164.

[21] B. Lee, M. Sharron, L.J. Montaner, D. Weissman, R.W. Doms, Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages, Proc. Natl. Acad. Sci. USA 96 (1999) 5215–5220.

[22] A. Heredia, A. Amoroso, C. Davis, N. Le, E. Reardon, J.K. Dominique, E. Klingebiel, R.C. Gallo, R.R. Redfield, Rapamycin causes down-regulation of CCR5 and accumulation of anti-HIV beta-chemokines: an approach to suppress R5 strains of HIV-1, Proc. Natl. Acad. Sci. USA 100 (2003) 10411–10416.

Please cite this article as: Vidita Choudhry et al., Increased efficacy of HIV-1 neutralization by antibodies at low ..., Biochemical and Biophysical Research Communications (2006), doi:10.1016/j.bbrc.2006.07.163.